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Natural killer cell deficiency in patients with non-Hodgkin lymphoma after lung transplantation

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KEYWORDS:
Lung transplantation;
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perforin

BACKGROUND: Post-transplant non-Hodgkin lymphoma (NHL) is a well-recognized complication of solid-organ transplantation, and pharmacologic suppression of adaptive immunity plays a major role in its development. However, the role of natural killer (NK) cells in post-lung transplant de novo NHL is unknown.

METHODS: Extensive phenotypic analyses of NK cells from patients diagnosed with NHL after liver or lung transplantation were conducted with multicolor flow cytometry. Polyfunctionality assays simultaneously assessed NK cell degranulation (CD107a) and intracellular cytokine production (interferon-γ and tumor necrosis factor-α) in the presence of NHL target cells.

RESULTS: The development of de novo NHL is linked to NK-cell maturation defects, including overexpression of NKG2A and CD62L and down-modulation of inhibitory killer immunoglobulin-like receptors and CD57 receptors. More importantly, in patients who developed NHL after lung transplantation, we observed a specific down-modulation of the activating receptors (NKp30, NKp46, and NKG2D) and a sharp decrease in perforin expression and degranulation against NHL target cells.

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CONCLUSIONS: Our results suggest that accumulation of abnormal NK cells could play a role in the outgrowth of NHL after lung transplantation, independently of the immunosuppressive regimen.
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The development of post-transplant lymphoproliferative disorder (PTLD) is the most serious life-threatening complication of immunosuppressive therapy after solid-organ transplantation (SOT) and contributes significantly to post-SOT morbidity and mortality.[1] The incidence of lymphoma is more than 10 times higher in these patients during the first 10 years after SOT than in the general population. This rate has remained remarkably stable during the past 2 decades, despite major changes in the immunosuppressive regimens used.[2] The risk of PTLD, however, varies according to the organ transplanted: it is greatest among lung recipients, ranging from 2.5% to 10%, compared with recipients of other organs; for example, the rate in liver recipients is 1% to 3%. This difference may be due to more intense immunosuppression in lung transplantations or to the large quantity of lymphoid tissue conveyed within the lung graft.[2,3] PTLD risk is highest in the first year after transplant and then decreases and rises again to reach a plateau that begins approximately 4 to 5 years after transplant.[1]

Non-Hodgkin lymphoma (NHL) is the most common PTLD and is often associated with Epstein-Barr virus (EBV).[4] EBV is a ubiquitous γ-herpesvirus that infects more than 90% of the adult population and becomes persistent through its latency in B cells.[5] A key factor in NHL pathogenesis is the immunosuppression-driven insufficiency of EBV-specific adaptive immunity. In this setting, immunomodulation, caused directly by the virus and by exogenous immunosuppressive drugs, impairs EBV-specific cytotoxic T-lymphocyte (CTL) responses critical to controlling the proliferative response and also impairs survival of infected cells.[6,7]

Consequently, research has largely focused on identifying the mechanisms that undermine adaptive immune control of EBV.[8] Reducing immunosuppression was the cornerstone of NHL treatment until recently, but response rates have varied markedly. Retrospective analyses of combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) show long-term disease control, but treatment-related mortality is as high as 31%.[9] Trappe et al[10] recently showed that the use of rituximab, followed by CHOP, improves the outcome of SOT patients with NHL.

Natural killer (NK) cells are generally assumed to contribute substantially to host anti-tumor immunity by destroying transformed cells and creating a local environment that suppresses tumor growth.[11] Differentiation of NK cells is driven by multiple cytokines and cell-to-cell interactions; it occurs in sequential steps, including the progressive loss of NKG2A expression and the acquisition of killer immunoglobulin-like receptors (KIR) and cluster of differentiation (CD) 57 as the cells evolve from immature $CD56^{bright}$ to more mature $CD56^{dim}$ cells.[12]

NK cells are controlled by an extensive array of inhibitory and activating receptors that may play a role in recognizing tumor cells that spare normal cells. The integration of these receptors determines whether NK cells become functional. KIRs, CD94/NKG2A, and immunoglobulin-like transcript (ILT)-2 account for most of the inhibitory receptors; they all recognize self-molecules of the human leukocyte antigen (HLA) class I repertoire, constitutively expressed on host cells. To destroy targets, NK cells also use several activating receptors, including NKG2D, DNAX accessory molecule-1 (DNAM-1), and the natural cytotoxicity receptors, including NKp30, NKp44, and NKp46.[13,14] Simultaneous interactions of some of these activating receptors on NK cells with their specific ligands on the target cells produce different intracellular signals that together dictate the quality and intensity of the effector NK-cell response.

Several lines of evidence also suggest that NK cells might play a key role in resistance to EBV-associated malignancies.[15,16] X-linked lymphoproliferative syndrome is a rare primary immunodeficiency characterized by extreme susceptibility to EBV infection that impairs the ability of NK cells to recognize EBV-transformed B cells.[17] Susceptibility to tumor development also increases after EBV-transformed B cells are transferred into severe combined immunodeficiency mice, reconstituted by NK-depleted cells.[18] Moreover, NK cell–mediated lysis of lymphoma via antibody-dependent cellular cytotoxicity might play an important role in the therapeutic activity of rituximab.[19] Saghafian-Hedengren et al[20] recently associated EBV infection with specific expansion of terminally differentiated NK-cell sub-sets, as previously observed in different acute and persistent infections, including hantavirus, chikungunya virus, and hepatitis.[21–23] We recently showed that the NK-cell expansion driven by cytomegalovirus (CMV) reactivation is associated with the development of specific de novo malignancies after orthotopic liver transplantation (OLT).[24,25]

Little is known about the role of NK cell surveillance during EBV latency or chronic EBV infection after SOT. In this study we investigated the sensitivity of NHL after OLT and lung transplantation (LT) to NK cell–mediated cytotoxicity. We demonstrate that SOT transplantation is associated with incomplete maturation of NK cells and a sharp decrease in perforin-dependent degranulation in patients who developed NHL after LT.

Methods

The local Institutional Review Board approved this study.

Patients and healthy donors

As summarized in Table 1, SOT patients with EBV-associated NHL were included at the time they were diagnosed (before any